Reference: *Biol. Bull.*, 141: 568–591. (December, 1971)

PARTICLE FEEDING IN NATURAL POPULATIONS OF THREE MARINE DEMOSPONGES

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The precise ecological roles of sponges remain undefined primarily because natural food materials have never been reliably determined for any member of the phylum. Early demonstrations of particle retention by sponges using non-nutritive materials (India ink, carmine, *etc.*) indicated that particle capture is accomplished by either amoebocytes or choanocytes, depending upon particle size and species of sponge studied. Both van Trigt (1919) and van Weel (1949), in providing nutritive but non-natural material to fresh-water spongillids, found that the site of particle capture was dependent on particle size. Large particles over 50 μ, unable to transverse ostia, were picked up by the surface epithelium; particles 5–50 μ were phagocytosed by archaeocytes and collencytes lining the inhalant passages; and particles smaller than 5 μ passed the prosopyles and were captured by choanocytes at the cell base or collar. Pourbaix's observations (1931, 1932a, 1932b, 1933a, 1933b) on living spongillids and on histological specimens of marine demosponges coincided with these findings and further indicated that capture sites may vary somewhat from species to species.

More recent works have been less conclusive and have cast doubt on the validity of the earlier works. Kilian (1952, 1964), in carrying out a detailed study of particle uptake using albumin-India ink and fresh-water spongillids, clearly substantiated the observations on van Trigt (1919) and van Weel (1949) in detail. His attempts to obtain lasting cultures of spongillids with live bacteria, however, failed. Because of this and his inability to demonstrate bacteria in intracellular food vacuoles, he concluded that bacteria could not serve as natural food materials for fresh-water sponges. Rasmont (1961, 1968), however, was able to obtain growth and maturation of spongillids on a diet of cleaned dead bacteria. His co-worker, I. Schmidt (Rasmont, 1968) observed that bacteria capture was effected by choanocytes. Simpson (1963, 1968) in attempting to substantiate the earlier observations of Pourbaix, was unable to find evidence of extra-nuclear DNA in choanocytes of the marine demosponge *Microciona prolifera*. He concluded that choanocytes probably do not serve as important sites of natural particle capture in this species. Works by Claus, Madri and Kunen (1967) and Madri, Claus, Kunen and Moss (1967), reporting to have demonstrated bacterial "removal" by *M. prolifera* held for long periods in immense cultures of *Escherichia coli*, have simply shown that *E. coli* numbers decrease in closed aquaria containing large masses of the proven bacteriostatic sponge *M. prolifera*. Capture and retention of bacteria by the sponges was not directly investigated, nor was it likely to have occurred in their experimental set-up.

All of the above works lack applicability for ecological analysis of marine demosponges in that they were qualitative, were performed with non-natural food materials usually under adverse laboratory conditions, or were based on work with the specialized group of fresh-water sponges. The present study is an attempt to resolve some of the contradictions in these observations and to provide a firm qualitative and quantitative assessment of the natural food materials of three species of marine demosponges.

Materials and Methods

Three species of Demospongiae were selected for investigation on the bases of (1) abundance and importance in the local ecosystem, (2) taxonomic distance between species and (3) morphology, utilizing specimens with single large oscula for ease of water sample collection. Each of the species selected is a dominant or important member of the lush Porifera fauna of the north coast of Jamaica. The work was carried out at the UWI-SUNY Marine Laboratory at Discovery Bay, Jamaica.

Tethya crypta (de Laubenfels, 1949, as *Cryptotethya*), S. C. Tetractinomorpha O. Hadromerida, is common throughout the eastern shallow protected areas of Discovery Bay, Jamaica. Within this habitat, the species is restricted to depths of −1 to −6 meters. All investigations of this species were made on a locally dense population situated at −3 m. The other two species, *Verongia gigantea* (Hyatt, 1875), S. C. Ceractinomorpha O. Dictyoceratida, and *Mycale* sp., S. C. Ceractinomorpha O. Poecilosclerida, comprise major components of the sponge-dominated fauna of the deep outer coral reefs. (The *Mycale* here, presently without a valid specific name, is that described by de Laubenfels, 1936, p. 116, but erroneously referred to as *M. angulosa*.) Although these two species coexist over a considerable portion of their ranges, −24 to −52 and −15 to −55 m, respectively, the center of abundance of *Verongia* on the deep fore reef is significantly deeper (−43 m) than that of *Mycale* at the junction of the fore-reef slope and the deep fore reef (−33 m) (reef nomenclature after Goreau and Wells, 1967). These two species show partial physical niche separation in this relatively nannoplankton-poor habitat.

In a typical higher demosponge, seawater streams slowly into the extensive inhalant surfaces, traverses a series of three successively finer, discrete filtration systems (1, dermal membrane, 2, inhalant canals and prosopyles, 3, choanocyte collars; van Gansen, 1960; Jørgensen, 1966), is re-collected by a system of converging exhalant canals and is ejected at high velocity from the vicinity of the sponge through the large exhalant osculum (or oscula). Essentially all exchanges of materials (food, respiratory gases, wastes, *etc.*) taking place between the specimen and the environment occur during transit of water through the canal systems. To investigate the natural exchanges of particulate organic material occurring in field populations of the three species noted above, samples of water were collected *in situ* from near the inhalant surfaces (ambient water) and from the oscular stream (exhalant water). The samples were individually analyzed for plankton by direct microscopy and for total particulate organic carbon (POC) by chemical analysis. The comparison of ambient and exhalant samples provides direct

assessment of the rates of filtration of plankton particles by type and size, as well as an overall assessment of the natural diets of the three species studied.

Water samples for direct microscopic analysis of plankton were collected during summer and winter in clean 25 ml polyethylene syringes. Exhalant water samples were obtained by carefully inserting the capped syringes into the exhalant stream below the margin of the osculum, taking care not to contact the specimen. The caps were removed while in the stream, the syringes slowly filled and the caps replaced while still in the stream. Ambient samples were taken near the inhalant surface of the same specimens immediately following collection of the exhalant samples. Within one hour of collection, 15 ml aliquots of each of the samples were filtered through 25 mm 0.22 μ Millipore type GS membrane filters at a suction of 0.3 atm or less (Holmes and Anderson, 1963). The filters were fixed in formalin fumes for 30 minutes, air dried, and stained in a membrane-filtered solution of 1% erythrosin-phenol for 1 hour at 60° C (Jannasch and Jones, 1959; Kriss, 1963). They were then rinsed in distilled water, air dried, and mounted in balsam under cover slips on microscope slides. A total of 30 such ambient/exhalant pairs of samples were collected and examined by direct microscopy.

Organic (red-stained) particles on each filter, within the size range able to pass the 50 μ diameter pores of the dermal membrane of these sponges (*i.e.*, having no more than one major axis greater than 50 μ), were recognized as 4 major fractions by size and morphology: (1) bacteria, (2) unarmored cells, (3) armored cells (fungi, diatoms, dinoflagellates, coccolithophores, *etc.*) and (4) detritus. The term "detritus" is used here in the narrow sense (see Bakus, 1969) to include only discrete, visibly resolvable, organic particles which are obviously non-living and which consist primarily of cellular debris and planar flakes. This is in contrast to the general, nonspecific, operational definition (see Jørgensen, 1966) for all supposed non-living material retained by glass fiber filters.

Two size classes of bacteria were recognized and enumerated separately in 5 randomly selected fields of each filter under oil immersion (1600 ×) for a total area scanned of 0.059 mm^2 or effectively 0.00356 ml of the sample. Although bacteria were small, 0.3–0.8 μ in greatest dimension, little or no practical difficulty was encountered in recognition due to the superior surface characteristics of the 0.22 μ membranes (in contrast to the previous 0.45 μ standard) and the general lack of similar-sized inorganic particles in these waters. Clumping of bacteria was found to be very rare at these concentrations and offered no problems to enumeration. Length and width of 25 bacteria of each of the 2 size classes were estimated against a 0.4 μ micrometer scale to $\pm$ 0.05 μ. Particle volume of the 2 sizes was calculated assuming a regular ovoid form.

All larger particle fractions (2–5 μ) were enumerated in a single continuous scan across a major diameter of each filter under high-dry 44 × objective (700 ×), covering a total area of 4.68 mm^2 or effectively 0.28 ml of the sample. After suitable familiarization with the spectrum of particle shapes of the local plankton, a system of 25 shape categories was developed for armored plankton groups. A relationship between length and particle volume was determined for each shape category (*e.g.*, volume of dinoflagellate $A = 0.335\ L^3$; *etc.*), based on length, breadth, and width measurements of 25 particles of each category and approxima-

tions to regular geometric shapes (for similar methods see: Jørgensen, 1966, Sec. 2.IIII; Mullin, Sloan and Eppley, 1966; and Strathmann, 1967). In scanning each filter, the length of every particle encountered was measured and its volume approximated on the basis of its shape category. Because of the poor quality of preservation, plasma volumes of diatoms could not be readily determined, and thus only cell volumes were obtained (see Strathmann, 1967, for a discussion of significance).

While the shape of armored cells is reliably maintained on filters, and the direct geometric procedure provides a suitable means for volume approximation, it is not suitable for unarmored cells. The diameter of the flattened ghosts of unarmored cells retained on the filters bears an unknown relationship to the volume of the original cell. For procedural reasons I have employed the assumption that the surface area of the ghost (both sides) reflects the total surface area of the flattened original cell pellicle and thus original cell volume = approximately $0.185 \times$ ghost diameter^3. If these cells do not spread laterally (*i.e.*, maintain constant cell volume as they encounter the filter surface), but instead simply collapse as the cytoplasm flows through the rupture at the point of first contact with the filter, then contraction of the pellicle would be severe, and the true cell volume may be 2 or $3 \times$ that estimated by the assumptions adopted here.

The organic carbon content of the 4 microscopically resolvable fractions (MPOC) was estimated for each sample from the above determined particle volume and from the relationships of carbon to volume (C/V ratios from other works). A general C/V ratio of 0.10 was employed for the bacterial fraction (Oppenheimer and Jannasch, 1962, use 0.05; ZoBell, 1963, uses 0.10; Jørgensen, 1966, uses 0.11). The C/V ratios applied to plankton cell fractions were adopted from Strathmann's (1967) work on analysis of phytoplankton cultures:

$$\text{for diatoms:} \quad \log C = -0.422 + 0.758 \ (\log V);$$
$$\text{for all other cells:} \quad \log C = -0.460 + 0.866 \ (\log V).$$

These size-dependent ratios were applied to each and every armored plankton cell encountered on each filter. Although an appropriate C/V ratio is unavailable for detrital material, a not unreasonable C/V ratio of 0.25 has been employed for this fraction on the basis of its generally accepted high cellulose content.

The procedures of enumeration provided mean raw counts of 141 and 406 bacteria per ambient sample of outer reef and bay waters, respectively. A total of over 14,500 bacteria were directly counted and assigned to class size. The high-dry scan covered an effective area of 87 fields and produced mean raw counts of the 3 fractions enumerated of 105 and 144 particles per ambient sample of outer reef and bay water, respectively. Size measurements and calculations of volumes and POC were made on a total of over 6500 individual particles of fractions 2–4. For individual fractions, mean counts for outer reef and bay water, respectively, were: unarmored cells, 64, 72; armored cells, 31, 47; detritus, 10, 17. Numbers of bacteria and totals for the 3 other fractions are considered to be highly dependable, within 20% of the true value for each sample. Counts of the rarer fractions—detritus and armored cells—are considered to be inaccurate because of the low numbers of cells, and may vary from the true value by a factor of 2 for any single sample.

Final calculations of POC content include not only true variations in the abundance of each fraction resulting from inherent patchiness and variations in seasonal abundance, but also include variations resulting from enumeration procedures, size measurements, and utilization of empirically derived C/V ratios. A reasonably accurate determination of the artificial variation attributable to handling and other procedures can only be obtained through replicate analysis of individual samples—which was not carried out.

Estimates of bacterial POC of any single sample are considered to be within $\frac{1}{3}$ to 3 × the true value, most of this attributable to possible error of size estimation. The POC values for unarmored cells may be $\frac{1}{10}$ to 3 × the true value for any given sample, due mainly to the assumptions of size and C/V ratio. Volume determination of armored and detritus particles is considered highly accurate, since size and shape are maintained throughout procedures. The POC values of these 2 fractions are still considered only to be within $\frac{1}{4}$ to 4 × the true value for any given sample primarily due to relatively large probable errors resulting from the random occurrence of large individual particles.

Although error of the estimate of POC content for any single fraction in any single sample is admittedly high, statistical treatment of adequate sample numbers compensates for sampling errors in determination of mean values with high accuracy (standard errors provided with data). Inasmuch as measurement and conversion errors are applied uniformly to both ambient and exhalant samples, the differences between samples—that is, effective filtration rates for specific particle fractions—are relatively independent of even these errors, as indicated by narrow confidence intervals supplied with data. These direct methods still remain the best available for quantification of plankton standing stock (Wood, 1968a).

In Jamaican waters zooplankton was essentially restricted to near-surface layers, the waters bounding the reef or bay bottom remained effectively free of larger particulate materials. Phytoplankton exceeding the size limits of the dermal pores of sponges (2 dimensions greater than 50 μ) were also scarce in bottom boundary waters—estimated from filter abundance to amount to less than 5% of the total plankton standing stock. No particles larger than 50 μ in greatest dimension were encountered in exhalant water samples. In all samples analyzed, the 4 particle fractions enumerated included at least 99.8% of the resolvable particulate material able to pass the dermal pores (by particle volume) and 95% of the total plankton. The aperture of the syringe collector was of sufficient diameter (2 mm) to preclude selective exclusion of particulate material present.

Water samples for chemical determination of particulate organic carbon (CPOC) were collected in cleaned 6-liter polyethylene bags fitted with 2–5 cm diameter stoppered closures. A stoppered bag was suspended by tripod above the specimen and oriented with the closure inserted into the oscular opening. The stopper was then removed and the bag was inflated by the low pressure of the exhalant stream of the specimen. Collection time for a 6-liter exhalant sample varied from 30 seconds or less with large specimens of *Verongia* to 15 minutes for small *Mycale* and *Tethya*. An ambient water sample was taken from the vicinity of the inhalant surface of the same specimen in an identical container during collection of every exhalant sample. When fully inflated, the exhalant

sample bag was capped while still in the exhalant stream and returned to the laboratory. After the bags were rinsed in distilled water, the 6-liter water samples were filtered through pre-combusted 4.25 cm Reeve Angel #934AH glass fiber filters (tested as superior in particle retention to standard Whatman type GF/C), and analyzed for POC following the "wet ashing" acid dichromate method outlined by Strickland and Parsons (1965). Extinction was measured on a Bausch and Lomb Spectronic 20 and POC calculated to $\pm$ 1 mgC/m^3.

Eleven such ambient/exhalant paired samples were collected and analyzed for CPOC. Aliquots of 8 of these pairs of samples were also analyzed by direct microscopic methods to determine differences in performance, if any, between the plastic bag and syringe collectors. Statistical tests of total particle volume and cell numbers of larger plankton fractions indicate there was no significant difference between ambient water samples collected with syringes or bags ($0.10 < P < 0.90$, Wilcoxan two sample statistic).

The basic difference in operation of the two collectors does, however, allow expectation of slight differences in exhalant samples. The syringe is basically a rigid, wiped-piston collector, and thus ambient water is potentially able to move by the rubber seal to compensate for compression of small gas bubbles within the syringe neck during descent. The action of drawing back the piston during sample collection also potentially allows some ambient water and thus particulate material to enter the barrel from the piston end. Exhalant water samples collected by syringe could be expected to be slightly contaminated with ambient water and indicate lower filtration rates than the bag samples, with an expected bias to small particle sizes. It appears that this does, in fact, occur, although the net contamination is slight (to be discussed below). Comparison of bag samples with samples collected in pre-cleaned glass-stoppered bottles indicated that the polyethylene itself contributed no detectable carbon to the samples within the limits of resolution of the analytical procedures.

The type of water samples available in this study—samples of seawater collected *in situ* before and after true single-pass filtrations—has not previously been employed in analysis of particle selection in any filter feeding organism. This study is, therefore, entirely free of the usual criticisms encountered in laboratory studies: (1) unknown feedback influences due to recycling of media in closed systems (*e.g.*, Jørgensen, 1949); (2) contamination of post-filtration samples with unknown quantities of unfiltered media (*e.g.*, Haven and Morales-Alamo, 1970); and (3) the universal problem of indeterminate influences of non-natural laboratory conditions.

Results

Available particulate organic materials

The distribution of particulate materials potentially available as food for the sponges studied here are presented in Table I in terms of particle numbers, particle volume, and organic carbon (MPOC, calculated). The data effectively represent means of approximately equal numbers of samples collected in late summer (September–October 1969) and late winter (January–March 1970), periods of maximum and minimum temperatures. All bay samples were taken from a restricted area at -3 m; outer reef samples were collected over the range -15 to

−52 m. All ambient samples analyzed by direct microscopy (30 syringe samples and 8 plastic bag samples) are incorporated. The relatively richer shallow bay waters contain approximately 1.7 × the particulate organic material (in volume or carbon) found in the clear outer reef waters. Water within the bay also main-

TABLE I

Available particulate material from analysis of ambient water samples

Particulate fraction	Bay (11 samples)	Outer reef (27 samples)	Stat. signif. P
Particle concentration—number/ml ± s.e.			
1 Bacteria	114,000 ± 21,600	39,740 ± 4,340	< 0.01
2 Unarmored cells	275.7 ± 32.7	227.3 ± 26.1	NS
3 Armored cells	166.7 ± 17.3	109.5 ± 11.6	< 0.01
4 Detritus	60.2 ± 9.3	35.7 ± 5.7	< 0.05
All non-bacterial particles	516.5 ± 47.0	374.8 ± 34.2	< 0.05
Particle volume—$10^3\mu^3$/ml ± s.e.			
1 Bacteria	5.72 ± 1.20	1.96 ± 0.21	< 0.01
2 Unarmored cells	42.67 ± 6.63	28.02 ± 3.41	< 0.05
3 Armored cells	12.03 ± 1.52	4.59 ± 0.56	< 0.01
4 Detritus	1.72 ± 0.39	1.03 ± 0.17	NS
Total all particles	62.24 ± 6.57	35.62 ± 3.51	< 0.01
Calculated organic carbon—mg/m³ (= 10^{-9}g/ml) ± s.e.			
1 Bacteria	0.571 ± 0.120	0.196 ± 0.021	< 0.01
2 Unarmored cells	6.74 ± 0.89	4.48 ± 0.51	< 0.05
3 Armored cells	1.60 ± 0.149	0.706 ± 0.081	< 0.01
4 Detritus	0.408 ± 0.089	0.258 ± 0.042	NS
Total all particles (MPOC)	9.336 ± 0.906	5.565 ± 0.530	< 0.01
Total range	(5.53 — 14.99)	(1.49 — 13.0)	
Chemically determined total POC—mg/m³ ± s.e.	(6 samples)	(5 samples)	
Total CPOC	85.95 ± 6.61	63.88 ± 3.27	< 0.01
Unresolvable POC***	75.70 ± 12.75**	55.00 ± 2.21	< 0.025

* Wilcoxan (Mann-Whitney) two sample statistic.
** Only 3 of the 6 samples were directly analyzed by microscopy.
*** Calculated as total CPOC minus MPOC.

tains significantly higher standing stock of each particle fraction except detritus for which differences between habitats are not statistically significant. Significant seasonal variations in abundance of specific plankton fractions do oocur but are beyond the scope of this report.

Bacteria (fraction 1), while present in moderate numbers, comprise only 4–10% of the biomass of available directly resolvable particulate material. Un-

armored cells (fraction 2), 5–50 μ in diameter, constitute the largest single resolvable fraction (70–80% of total) in both habitats in all seasons. These cells are recognized on filters as flattened cell ghosts with small central nuclei surrounded by mitochondrial particles. Recognizable chloroplasts are lacking. The fraction undoubtedly includes the naked flagellates, generally the major element of plankton biomass of tropical waters (Hulburt, Ryther, and Guillard, 1960; Bernard, 1963; Mullin, 1965; Wood, 1968c).

The armored cells (fraction 3) constitute 13–20% of the biomass of available organic material. Two small classes of heavily armored particles, 2×2 μ and 3×4 μ, are present in high numerical abundance ($10^4 - 10^5$ cells/liter), but account for only 5–20% of the total armored cell fraction. These are similar to those reported in the Sargasso Sea (Hulburt, Ryther and Guillard, 1960) and the Mediterranean Sea (Bernard, 1963), and probably include several species of fungi and resting capsules of blue-green algae. Diatoms, dinoflagellates and coccolithophores alternate in abundance seasonally but maintain a relatively constant total. At least seven species of diatoms among the genera *Pinnularia, Nitzschia, Coscinodiscus* and *Licmophora* are present in both habitats, with greatest dimension of 5–100 μ. Dinoflagellates and coccolithophores, varying from 5–20 μ in greatest dimension, are dominated by representatives of *Gymnodinium* and *Oxytoxum* (from Wood, 1968b). No single armored species is overwhelmingly dominant in any single sample.

Detrital particles (fraction 4), including skeletal debris of plankton and flat flake-like structures, account for only 3% of the total resolvable material by volume and 5–10% by calculated carbon.

Chemical analysis of total particulate organic carbon (CPOC) was carried out only on late winter samples. Both bay and outer reef waters contain POC values within the range reported for open waters of the Gulf of Mexico by Fredericks and Sackett (1970) and are considered to be typical, nutritionally-poor tropical waters. The results of the two methods of analysis indicate that fully 86–88% of available carbon of these samples cannot be accounted for by microscopically detectable particulate fractions—including detrital material. By convention such differences between total CPOC and plankton POC, normally encountered in analysis of seawater throughout the world, are attributed to detrital material (Jørgensen, 1966). In the samples of Jamaican waters studied here, detrital material is not sufficiently abundant to account for this discrepancy, even if a C/V ratio of 1.0 is employed for this fraction. On the basis of the available information (and other evidence to be discussed below) it appears that the major portion of the functionally particulate organic carbon in Jamaican water is present as material unresolvable by direct microscopy (URPOC).

Retention rates by particle type and size

The per cent efficiency of retention,

$$\frac{\text{ambient-exhalant}}{\text{ambient}} \times 100$$

is shown in Table II and Figure 1 for each particle fraction for each species of demosponge investigated. Means and 95% confidence limits of the means were

approximated using arcsin percentage transformation and student's t distribution (Owen, 1962). Levels of significance of differences in retention between species of sponges for given particle fractions, and conversely between particle fractions for a given species of sponge, were calculated using the Wilcoxan (Mann-Whitney) two sample statistic (Owen, 1962).

Bacteria were retained by all three species at high efficiency-mean of the three species is 96.1% by number of particles and 96.4% by calculated volume or carbon (rates of retention of carbon and volume are identical since a single, size-independent C/V ratio was employed for this fraction). Retention differences between species are insignificant for the complete fraction, as well as for and between the

TABLE II

Retention efficiencies of natural particulate organic fractions by three sponges

Fraction	Mean % retention (95% confidence interval of the mean)		
	M. sp.	*V. gigantea*	*T. crypta*
Bacteria	10 samples	7 samples	11 samples
Bacteria			
By number	96.9 (95.0– 98.4)	94.8 (91.3– 97.5)	96.5 (94.7– 98.0)
By volume	97.6 (95.6– 98.9)	95.1 (90.2– 98.4)	96.2 (94.0– 98.0)
Other fractions	14 samples	13 samples	11 samples
Unarmored cells			
By number	80.4 (73.9– 86.2)	82.5 (76.8– 87.6)	94.4 (91.0– 97.0)
By volume	88.2 (83.2– 92.3)	88.4 (83.9– 92.2)	91.7 (82.4– 97.7)
Armored cells			
By number	41.2 (30.3– 52.5)	38.7 (19.3– 60.2)	66.0 (53.4– 77.5)
By volume	64.5 (44.9– 81.8)	77.0 (61.7– 89.4)	80.1 (64.5– 92.0)
Detritus			
By number	−172.3 (+67.9– −412.5)	−186.4 (+32.2– −405.0)	−148.5 (−2.7– −294.3)
By volume	−51.7 (+35.7– −140.1)	−113.5 (−11.8– −215.5)	−82.8 (+12.8– −178.4)

smaller (0.028 μ^3) and larger (0.151 μ^3) size classes of bacteria ($P > 0.10$ in all cases). Within the ranges of bacterial concentration encountered, retention rates were found to be independent of abundance.

During a one-month period in mid-winter (16 January–17 February 1970), bacterial retention of both outer reef species, *Mycale* and *Verongia,* dropped far below the normally high 96% levels, while retention rate of other plankton fractions was unaffected (Fig. 2). This partial failure of only the bacterial capture system of both outer reef species, while retention of the bay species *Tethya* remained unchanged, suggests that the cause was a local shift in the composition of the bacterial fauna. The lack of change either in morphology of bacteria or abundance ratios of the 2 size classes suggests that the species composition of bacteria did not undergo a major shift at this time. The action of normal winter storms during and preceding this period had, however, caused heavy general mortality of the outer reef sponge fauna, leaving considerable numbers of damaged and decomposing sponges throughout the fore-reef slope. Bacteria, certainly utilizing these sponges as a nutritional substrate, may have incorporated significant

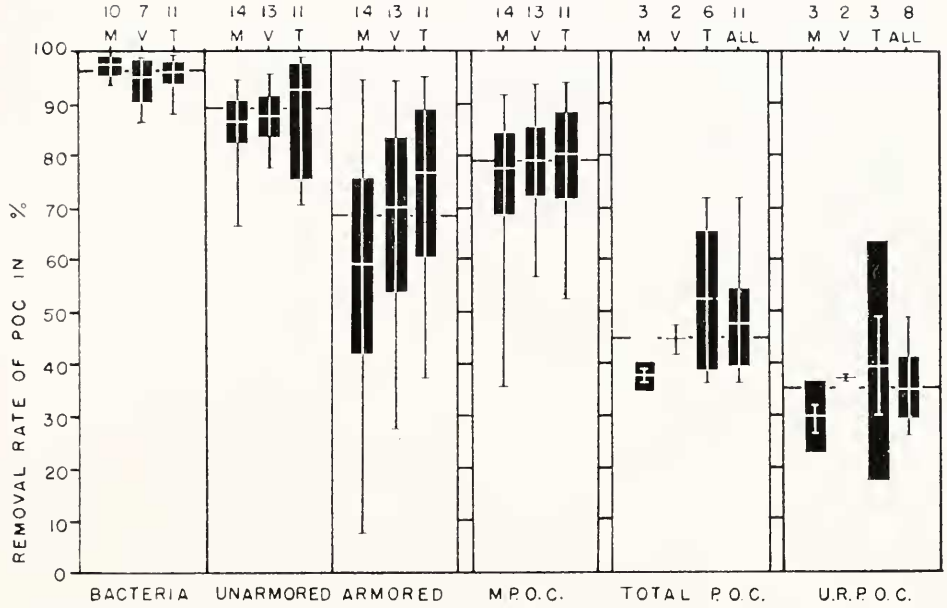

FIGURE 1. Per cent removal rates for various POC sources. Number of samples and sponge species are designated above the graph: M = *Mycale* sp.; V = *Verongia gigantea;* and T = *Tethya crypta.* Total range of samples is represented by the vertical line, mean by the horizontal (white) line, and 95% confidence interval of the mean by the solid bar (where calculable). The mean of the three sponges is shown as the broken horizontal line across each fraction interval. Range, mean and confidence intervals for all available samples of total POC and URPOC are shown to the right of these intervals.

amounts of distinctive sponge sterols (Bergmann, 1949) in surface membranes, which could have elicited rejection of these bacteria by the healthy, filtering sponges.

As has been previously indicated, the retention rates for bacteria may be slightly underestimated above due to partial failure of the syringe sample collector.

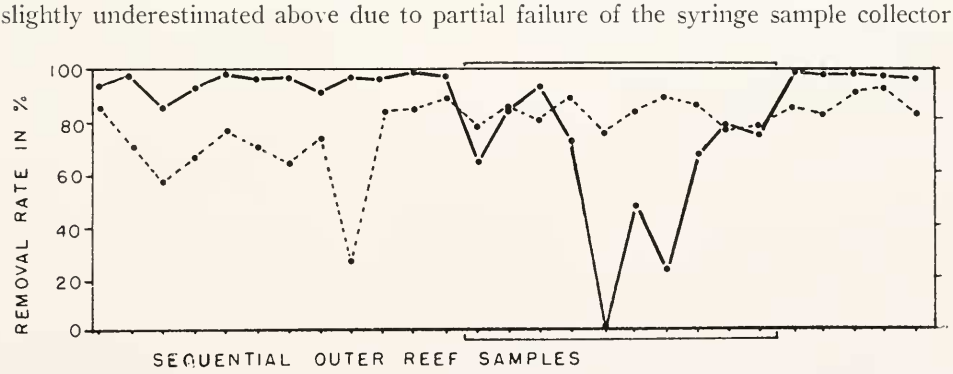

FIGURE 2. Mid-winter failure of bacterial retention by both outer reef sponges. The removal rates of bacterial POC (unbroken line) and all other MPOC (broken line) are shown for sequential water samples collected on the outer reef. Failure of bacterial retention (within brackets) occurred in both species, while retention of other fractions remained at normal levels.

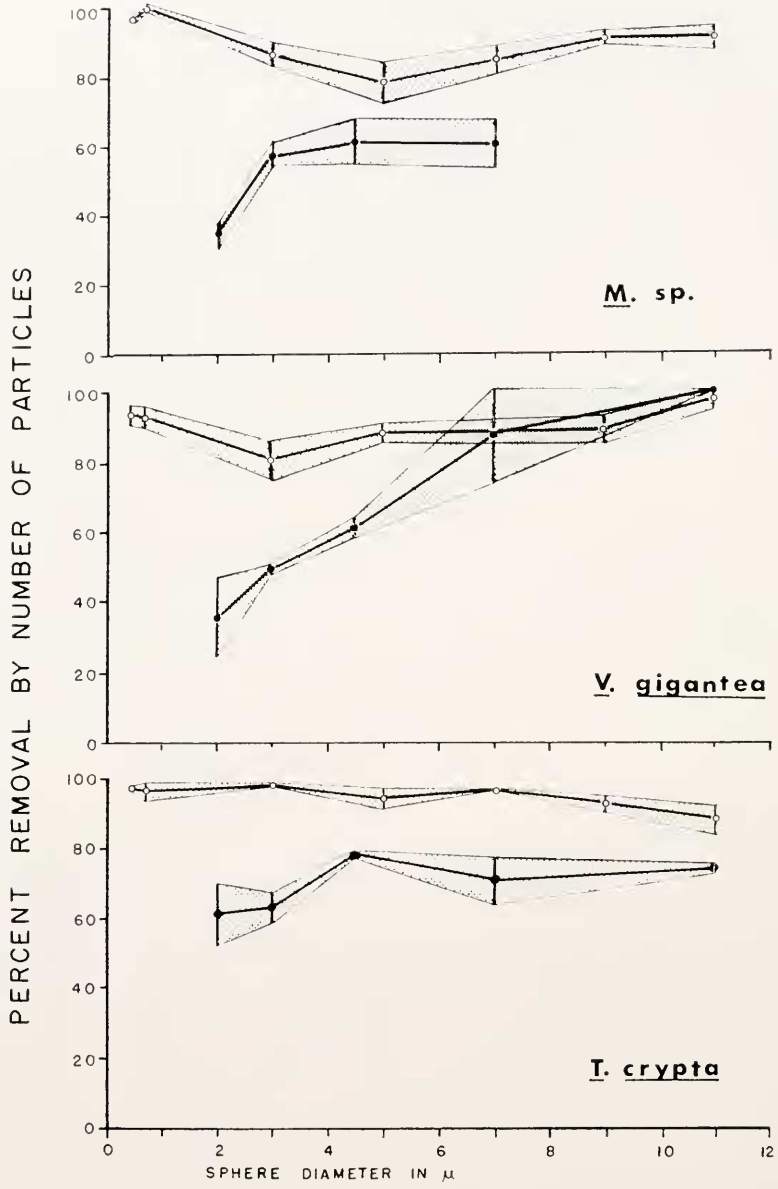

FIGURE 3. Relationship of particle size to retention rates for resolvable plankton fractions. Particles within fractions have been grouped by volume expressed here as diameter of sphere with equal volume. All winter and all summer samples have been grouped to provide adequate numbers of particles (> 30) within each size interval resulting in only 2 points for each grouping. Armored cells are shown in the lower block-area of each species, unarmored cells in upper. The unarmored fraction has been extended to the left, connecting with the two bacterial size classes.

Samples collected by plastic bags, which are free of the operational bias of syringes, show a mean retention rate of 98.1% by volume or carbon (8 samples). The probable contamination of exhalant samples by ambient water passing the plunger seal amounted to only approximately 1.7% of the sample volume.

Unarmored cells (fraction 2) are retained by all three species at high efficiency-means for the three species are 86.5% by numbers of cells and 89.3% by calculated cell carbon (Table II, Fig. 1). Retention rate by cell carbon for this fraction is significantly higher ($P << 0.001$) in *Tethya* than in the other two species, which do not differ ($P > 0.10$). The retention rate for unarmored cells by carbon is not significantly different from that of bacteria for *Tethya* ($P > 0.10$), while *Mycale* and *Verongia* both retain unarmored cells at significantly lower efficiencies than bacteria ($P < 0.01$). All three species show little evidence of size selectivity within the unarmored fraction (Fig. 3).

Armored cells (fraction 3) are retained at moderate rates by these three sponges—mean for the three species is 48.7% by cell numbers and 68.7% by cell carbon (Table II, Fig. 1). Due to high variability of individual samples (low particle counts) and to slightly different composition of the armored cell fractions in the two habitats, significant differences between the three sponges vary with analysis. By cell numbers, *Tethya* retains armored cells at signficantly higher rates ($P < 0.01$) than the other two species which do not differ ($P > 0.10$). By volume or cell carbon, a more valid estimate of potential food value, no significant differences are found between the three species in retention of this fraction. By carbon, all three species retain the armored fraction at significantly lower rates than the unarmored fraction ($P < 0.01$ in each case) and bacterial fraction ($P < 0.001$ in each case).

Within the armored fraction definite evidence in size selectivity is shown by two of the three species (Fig. 3). Smaller 2 μ diameter armored cells, within the size range of the prosopyle openings serving the flagellated chambers, are retained at very low efficiencies—20–45%—by both *Mycale* and *Verongia*. *Mycale* and *Tethya* exhibit a fairly constant rate of retention in larger armored particles, while *Verongia* retains larger cells with increasing efficiency—nearing 100% retention in larger effective cell sizes. Possible mechanisms for these differences will be discussed below.

Each of the three species of sponge exhibits a net production of detrital material (fraction 4) (Table II). Exhalant water samples contain approximately 2.7 × the number particles and 1.8 × the carbon content (calculated) of corresponding ambient water samples (means of the three species). Because of extreme variability in abundance of ambient detrital material, and the variability in production/retention values of individual sample pairs, confidence in the level of production of this material is very low. Due to the same variability, no statistical differences were found between the three species. In each case, the production of this fraction contrasts sufficiently with the retention of the other three fractions to obviate statistical demonsration of differences. The detrital material produced probably consists of incompletely digested cellulose walls and cell debris from the three major fractions of plankton particles. As there is no reliable way to distinguish newly produced detrital material from that taken in

with ambient water, the extent to which available detrital particles are utilized for food by these sponges cannot be determined with the methods employed.

All microscopically resolvable particulate material able to pass the 50 μ dermal pores is retained at fairly high rates by all three species (Table II, Fig. 1) —mean of the three species is 79.0% by calculated carbon content. This data includes the 4 fractions treated above and a minor component of miscellaneous particles not assignable to those fractions (filamentous blue-green algae, small fungi, *etc.*) The retention rates by numbers of particles (omitting bacteria) are low (mean = 41.9% for the three sponges), due primarily to the net production of large numbers of detrital particles which do not greatly lower volume or carbon values. The overall rates of retention for all particulate material do not differ significantly between the species ($P > 0.10$ in each case).

TABLE III

The net diets of three sponges in terms of POC removed from each particulate fraction per unit of seawater filtered

Fraction	POC source—mg/m³—s.e. (number of samples)			% of total POC retained (mean of three species)
	M. sp.	*V. gigantea*	*T. crypta*	
Bacteria	0.171 ± 0.037 (10)	0.137 ± 0.035 (7)	0.546 ± 0.116 (11)	0.89
Unarmored cells	3.77 ± 0.66 (14)	4.18 ± 0.75 (13)	6.07 ± 0.78 (11)	16.2
Armored cells	0.386 ± 0.078 (14)	0.622 ± 0.135 (13)	1.19 ± 0.16 (11)	2.4
(miscellaneous)	*	*	0.008 ± 0.002 (11)	0.007
Detritus	−0.042 ± 0.077 (14)	−0.117 ± 0.093 (13)	−0.382 ± 0.197 (11)	—
Total MPOC	4.29 ± 0.73 (14)	4.82 ± 0.77 (13)	7.43 ± 0.86 (11)	19.5
URPOC	15.81 ± 1.06 (3)	21.28 ± ** (2)	32.28 ± 6.30 (3)	80.5
Total POC	20.1	26.1	39.7	100.0

* Negligible.
** Only 2 samples available.

Chemically determined total CPOC

Comparison of CPOC content of the 11 ambient/exhalant pairs of large water samples indicates that total "particulate" organic carbon is retained by these sponges at low efficiencies (Fig. 1)—the mean of the three species is only 44.7%. The three sponges show no statistically valid differences, as expected from the small numbers of samples. The mean retention rate of all 11 paired samples is 47.7%, with a reasonably small 95% confidence interval for the mean (Fig. 1).

It has been suggested above that the major portion of chemically measurable POC (*i.e.*, retained on the glass fiber filters) in ambient water samples is not accountable as microscopically detectable plankton particles, but apparently exists as non-discrete non-resolvable material. The filtration rates derived for the 4 microscopically resolvable fractions and for total CPOC are consistent with this assumption, and provide supplementary evidence for the existence of the URPOC fraction (to be discussed in detail below).

The rates of retention of the preponderant fraction of available carbon as URPOC material were obtained from 8 of the large sample pairs collected in late winter. The calculated amounts of MPOC were subtracted from CPOC, providing estimates of URPOC of ambient and exhalant samples. Retention rates of URPOC are low (Fig. 2), mean of the three species is 35.2%. As only 2 or 3 sample pairs are available for each species, differences between species cannot be

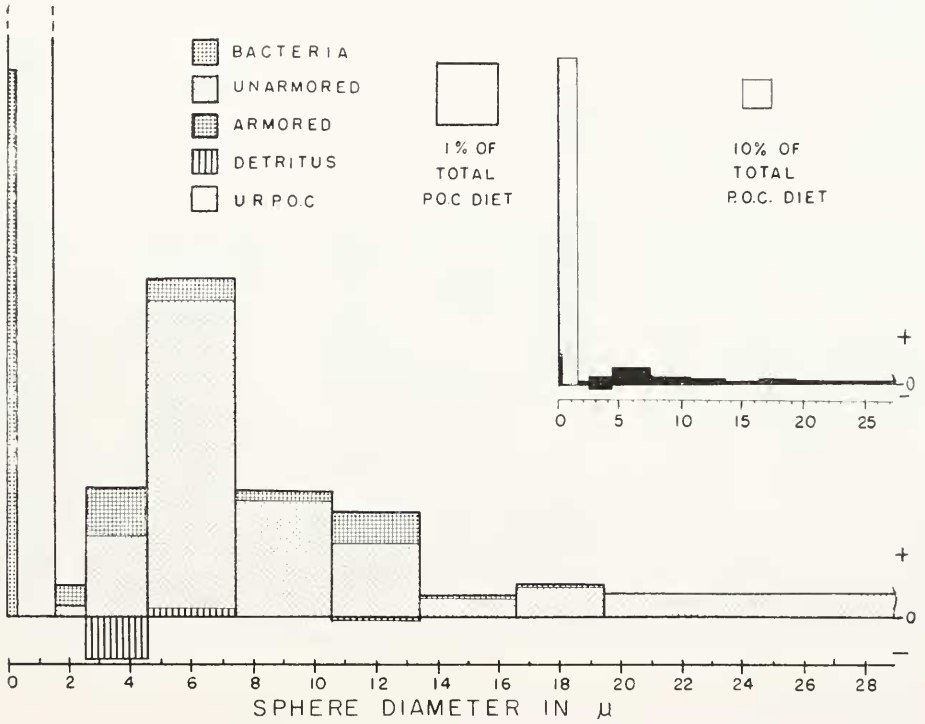

FIGURE 4. Mean size distribution of the dietary POC for three marine Demospongiae. All particles surveyed in the samples of each species have been grouped by volume interval (expressed here as diameter of sphere with volume equal to interval mid-point). Total POC content of each size interval was summed for each fraction and means of the three species are expressed as area of the interval block. Net retention and production are respectively shown above (+) and below (−) the zero line. URPOC is restricted to the interval between 1.5 and 0 μ effective size (see discussion). The total contributions of URPOC and MPOC are shown in the reduced inset (upper right).

statistically analyzed. The mean retention of URPOC for all 8 samples is 35.0% with a 95% confidence interval of 29.4–40.8%. The differences between retention of this URPOC material and all MPOC fractions is significant ($P < 0.001$).

Net POC diet of sponges

The estimated mean sources of dietary POC for the three sponges over the entire year (all samples) is presented in Table III. Because of its relatively

high abundance, the URPOC fraction provides by far the major carbon source. The three plankton fractions, although retained at high efficiencies by all three species, contribute a minor proportion, due to their relatively low concentrations in both habitats.

The size distribution of retained, and presumably utilized, POC within each fraction has been analyzed for each species, the mean of the three is provided in Figure 4. Particle size is represented on the abscissa as diameter of a sphere of equal volume. Particles are grouped in intervals of sphere diameter of: 1.5–2.5 μ, 2.5–4.5 μ, 4.5–7.5 μ, *etc.* Calculated POC is represented by area within each size interval for each fraction. Most retained MPOC consists of particles between 8.2 and 1,290 μ^3 in volume, or equal to spheres of 2.5 to 13.5 μ diameter. The reasonable restriction of URPOC to material less than 1.5 μ in effective diameter (to be substantiated below) renders the overall size distribution of dietary POC conspicuously bimodal (Fig. 4, insert).

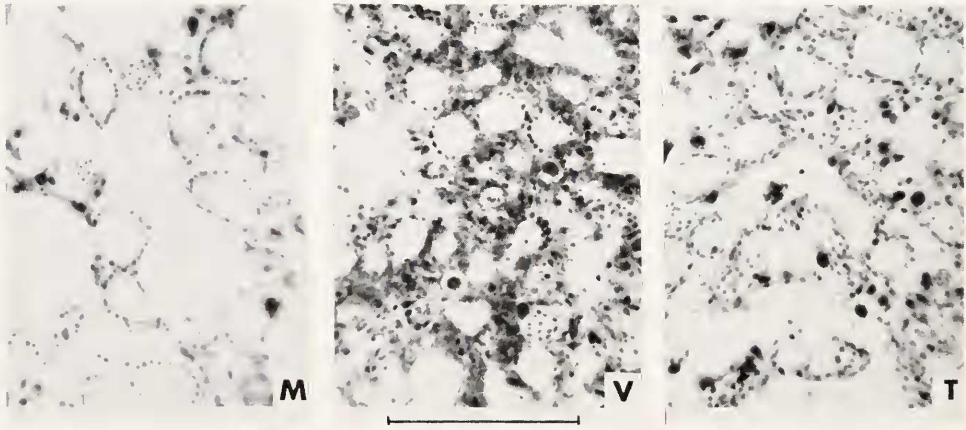

FIGURE 5. Histological sections of the three demosponges studied. Specimens have been identically processed for each species: *in situ* preservation in Bouin's fixative, 8 μ paraffin sections processed through Masson Trichrome stain, modified (Humason, 1962, page 154). (M = *Mycale* sp.; V = *Verongia gigantea;* T = *Tethya crypta;* 246 ×; scale = 100 μ).

Relationship of particle retention to anatomy

The slight differences in retention between plankton fractions (Table II, Fig. 1) and within the armored fraction (Fig. 3), may be attributable to the presence of plankton armor and to anatomical differences between sponges. Wandering amoebocytes, after picking up larger particles (> 2–5 μ in diameter) in inhalant canals, have been shown to carry out the normal pattern of digestion within phagocytic vacuoles, migrate to exhalant canals, and liberate the undigested contents of vacuoles into the post-chamber exhalant stream (van Weel, 1949; Kilian, 1964). The differences in retention rates between unarmored and armored cells is almost certainly attributable to the resistance to digestive enzymes conferred by cell armor (cellulose and siliceous tests appear to be equally resistant).

If success of digestion is furthermore a function of time of exposure, the patterns of retention within the armored fraction may be explained by differences

in canal systems and tissue density between the three sponges (Fig. 5). Migration of particle-laden amoebocytes from inhalant to exhalant canal systems is expected to be rapid in *Mycale*, as physical distances are short and tissue density is low. In *Verongia*, with greater distances between canal systems and higher tissue density, migration time is expected to be extended. Amoebocytes ferrying larger particles are expected to encounter proportionally greater difficulties in *Verongia* and digestive susceptibility is expected to be a function of particle size—as found above. In *Mycale* and *Tethya*, tissue density apparently presents no appreciable obstacle to cell migration, and susceptibility to digestion in armored cells is not expected to be size-dependent—also as found above (Fig. 3).

The low retention rates of small (2 μ diameter) armored particles by *Mycale* and *Verongia* are almost certainly due to passage of large proportions of these cells through the prosopyles (2–4 μ) and flagellated chambers (between adjacent collars—not between microvilli) into the exhalant stream. The high rate of retention of these small particles by *Tethya* is not explainable by morphological differences.

Discussion

The results of this study indicate that most of the total available POC in Jamaican waters, and the dietary POC of the three sponges, consists of material retainable by glass fiber filters, but not resolvable by direct microscopy. It is not assignable to the detrital fraction as narrowly interpreted here. The existence and possible nature of this URPOC material (the deficit between CPOC and MPOC) may be inferred from close inspection of the data. Can this material be attributed to erroneous estimation of plankton fractions? Bacteria, armored cells and detrital fractions each comprise less than 2% of the total available POC. If procedural errors on carbon estimation were as high as 300 to 400% (maximal estimated for single samples) for these fractions together they could still account for only a small portion of the carbon deficit. The gross differences between retention rates of these three discrete fractions and the URPOC material increases the unlikelihood of this explanation.

Because of the inherent uncertainties in enumeration, size determination, and volume to carbon conversion of unarmored cells, only this fraction of the microscopically resolvable material could potentially contain the unaccounted for carbon. To account for the missing carbon of ambient samples in this, the largest of the resolvable fractions, the true POC content of this fraction would be at least 10 $\times$ the value calculated and more probably 50 $\times$ since these unarmored cells are expected to rupture upon contact with the filter, losing much of their carbon. The high retention rate of the unarmored fraction by all three species, however, contrasts sharply with the low rate of the missing carbon. Since procedural errors are expected to be equally applied to both ambient and exhalant samples, rates of retention of the unarmored cells and missing carbon would be expected to be equal if transformations do not take place during passage through the sponges. It is obvious, then, that if the unarmored fraction is hypothesized to contain the carbon deficit of ambient samples, it cannot at the same time account for the proportionally different deficit of exhalant samples. The unarmored fraction can contain the ambient carbon deficit only if it is further postulated

that these cells are broken up or partially degraded in passing through the sponge. This would result in transformation of at least 70% of the carbon of the ambient unarmored fraction into material which is still retained by the glass fiber filters, but which is unresolvable by direct microscopy in the exhalant samples—URPOC material by definition.

The retention rates and POC calculations of each fraction thus allow only two alternative explanations for the carbon deficits: (a) the missing carbon of both ambient and exhalant samples exists as URPOC material, distinct from all resolvable fractions, or (b) the missing carbon of ambient samples is included in the unarmored fraction and is transformed in exhalant samples into material indistinguishable from the proposed URPOC material. Further information is available to aid in choosing between these alternatives. The extensive sponge fauna of the fore-reef slope habitat turns over (cycles) the 2-meter layer of water in contact with the reef each hour (from quantitative analysis of *in situ* pumping data and estimations of standing biomass of this habitat—Reiswig, unpublished). The high rate of transformation of carbon from unarmored to URPOC material postulated in alternative (b) would rapidly increase proportionate abundance of the URPOC material of ambient water. Thus the state of ambient water hypothesized in alternative (b) is dynamically unstable and rapidly would be expected to shift to the state hypothesized in alternative (a). Because alternative (b) requires assumptions of immense errors of at least an order of magnitude in calculation of unarmored POC and requires acceptance of a dynamically unstable state which appears to lead directly to the alternate assumption, the (a) hypothesis is considered to be far more likely to represent true conditions and as such has been accepted. The carbon deficit of both ambient and exhalant samples is considered to reside in a POC fraction defined as URPOC material.

The low retention of URPOC by all three sponges, and the lack of resolution of this material by light microscopy, suggests that it is quasi-particulate—*i.e.,* it is indeterminate and variable in size and shape. It is able to pass the discrete, planar filter of the choanocyte collar (0.1 μ slits) at significant rates. The effective size of URPOC is apparently within the size range of bacterial particles. Two different brands of glass fiber filters, precombusted under identical conditions, retained URPOC and bacteria at different rates. Reeve Angel #934AH filters retain 31% more total POC and 33% more of the smaller sized bacteria than the Whatman GF/C filters (means of 2 pairs of analysis), indicating that the URPOC material in these water samples has a mean effective size of approximately 0.3 μ.

On the basis of this study, the URPOC material is then hypothesized to consist of the larger size range of a continuous spectrum of quasi-particulate organic aggregates extending from isolated truly dissolved molecules to discrete detrital flakes at extremes of the range. The portion of this spectrum recognized as URPOC is defined by a lower size limit which is dependent only upon the characteristics of the glass fiber filters used for its collection—an operational and highly non-specific definition. The material is therefore hypothesized to exist in a state of physico-chemical equilibrium with truly dissolved material, from which it is generated and from which it is separated only by an arbitrary effective pore size.

Sheldon, Evelyn and Parsons (1967) have shown that organic particles are generated in standing seawater after filtration, although the formation of these particles was not proven to be independent of bacterial action. They hypothesized that the particles were bacteria, but were unable to provide incontrovertable evidence that such was the case (used the Coulter counter and plating methods, but did not make direct bacterial counts). Their particles could therefore easily be identical to the URPOC proposed here. Mullin (1965) reported that 45% of available POC of Indian Ocean waters resided in the $< 10\ \mu$ size fraction—consistent with the URPOC material proposed here. In coral reef habitats the shallow water coral-zooxanthellae communities produce a considerable excess of organic material (Kanwisher and Wainwright, 1967), probably as free polysaccharides. This may be the source material for production of URPOC in Jamaican waters. In temperate coastal regions, the products of algal communities (Bakus, 1969) may produce a similar material, although masked in these waters by the abundance of phytoplankton and resolvable detritus. The numerous reports of carbon deficits between CPOC and plankton POC (= MPOC) in marine waters throughout the world (see Jørgensen, 1966, for a review) suggest that the abundant URPOC found in Jamaican waters may reflect a general characteristic of all marine waters.

The amounts of total POC retained by these three sponges are below estimated needs for maintenance of the filtration/pumping machinery (50 mgC/m^3) and far below estimated levels required to sustain growth and reproduction (250 mgC/m^3) (Jørgensen, 1966). It will be shown elsewhere that the total natural POC demonstrated here to be retained in field populations of sponges does meet all energetic needs for two of the species—the third, *V. gigantea*, is exceptional in its requirement for dissolved organic carbon and in its possession of a vehicle for capture of DOC, symbiotic bacteria. Jørgensen's estimates are excessively in error as regards natural sponge populations, primarily because data available were inappropriate: oxygen utilization and pumping efficiencies were from very few laboratory studies, and energy conversion efficiency was taken from studies of Crustacea and Mollusca.

The results obtained here are entirely consistent with those of Pütter (1914) in which he found available plankton insufficient to satisfy dietary requirements of the sponge *Suberites massa*. Pütter hypothesized the capture and use of dissolved material by the sponge to account for the discrepancy. URPOC material, if abundant in Mediterranean waters as suggested by the differences between total POC and plankton (Sushchenya, 1963), would likewise have provided the missing carbon source and at the same time satisfy the requirements of Pütter.

In spite of the quantitative importance of URPOC in the diet of Jamaican sponges studied here, it provides little information on the particle capture systems functional in these sponges. Patterns of retention of discrete particulate fractions of the plankton, however, do indicate mechanisms of particle capture and transport. The bimodal pattern of particle retention (Figs. 1, 3 and 4) suggests that two independent systems of particle capture are functional in field populations of the three species studied here. This agrees in detail with the conclusions reached by van Trigt (1919), van Weel (1949) and others.

The primary capture system, involving particles greater than 2–5 μ, is attributable to the amoebocytes lining the inhalant aquiferous system. These cells apparently capture particles directly or phagocytose particles caught in the progressively narrower canals. This capture system must operate continuously and unselectively to prevent occlusion of the aquiferous system by suspended particles, a conclusion reached by previous workers using non-nutritive particles. The necessity for constant elimination of particles requires either a continual migrational cycling of amoebocytes from inhalant to exhalant systems, or constant physical flux of canal systems moving in space to bring excurrent canals into contact with trapped particles, or both. Evidence by van Trigt (1919), van Weel (1949), and Kilian (1952) favors the existence of amoebocyte cycling, while time-lapse cinematography of spongillids (Kilian, 1964) indicates that canal migration may be important in young sponges which have not yet developed dense populations of amoebocytes. The behavioral complexities shown by *Tethya* and *Verongia* (Reiswig, 1971) indicate that canal reorganization may be taking place in these sponges during cessation of pumping activity.

The retention differences noted between and within plankton fractions are attributable to variations of anatomy and thus to proposed differences in amoebocyte cycles, as indicated earlier (Fig. 5). More importantly, habitat restrictions are also partially explainable on this basis. *Verongia,* with a slow and easily saturated amoebocyte cycle, is expected to be less able to cope with heavy sediment loads. The species is restricted to the clean-water habitat of the outer reef. Even here it suffers serious decrease in pumping rates during increased turbidity caused by winter storms in spite of hypothesized reorganization and cleansing of canals during pumping cessation. *Mycale,* maintaining rapid amoebocyte cycles in a very loosely organized tissue reticulation, is not only able to sustain activity on the outer reef during winter storms, but is able to maintain successful populations within the sediment basin of Discovery Bay, a habitat devoid of the dense-tissue sponges.

By virtue of its loose architecture, *Mycale* appears to be able to maintain activity under a variety of conditions of turbidity, and thus invade many habitats from which more densely organized sponges are restricted, but as a corallary, a large portion of potential food as armored cells must necessarily be sacrificed via rapid particle cycling.

The situation of *Tethya* is intermediate and more complex. The diurnal cycle of pumping cessation shown by this species (Reiswig, 1971) suggests that particle capture may occur during daytime activity but digestion may extend throughout early morning cessation. The time of exposure of armored cells to digestive enzymes would be increased and utilization of the armored fraction high—as found. *Tethya* is thus able to exist in a habitat of high particle concentration, and at the same time maintain high rate of utilization of the armored cell fraction, but in this case sacrifices a significant portion of the time of filtration.

The relationships found in these three sponges between architecture and habitat restriction appear to be general for the phylum Porifera. The restriction of dense tissue Keratosa to clear water, tropical and subtropical habitats is world-wide and may be accounted for by the concomitant susceptibility of these sponges to high particle concentrations. The dominance of low tissue density

sponges (*e.g.*, Haplosclerida, Poecilosclerida and Halichondrida) in temperate coastal waters with high particulate load is consistent with this scheme. It is further hypothesized that when sponges of intermediate tissue density (*e.g.*, Hadromerida) are present in coastal waters of high turbidity, behavioral complexities augmenting normal amoebocyte cleansing will be found to exist as in *Tethya*.

The secondary particle capture system, responsible for the uptake of bacteria, some of the minute 2–4 μ elements of the nannoplankton, and very probably the large URPOC fraction, consists almost certainly of the choanacytes. Capture of bacteria and bacterial-sized particles by choanocytes has been repeatedly reported by direct observation (van Trigt, 1919; Pourbaix, 1933a; van Weel, 1949; Kilian, 1952; Rasmont, 1968). Since inhalant canal systems vary between the three sponges, but the ultrastructure of the choanocyte collar is presumably uniform throughout the phylum (0.1 μ spacing between microvilli), the nearly constant rate of bacterial filtration found here almost certainly occurs at the collar surfaces. All Porifera probably remove bacteria at high efficiencies. It is interesting to note also that the immense population of symbiotic bacteria harbored by *Verongia gigantea* (and other Verongiidae—Lévi and Lévi, 1965; Vacelet, 1967) does not significantly affect net bacterial retention rate by this species. This intra- and inter-cellular population must be physically retained with high efficiency and is probably maintained at a controlled rate of population growth equal to that of the sponge.

The inability of Simpson (1963) to find extranuclear DNA in choanocytes of *Microciona prolifera* is explainable by the high density of choanocytes, the relatively low availability of bacteria per choanocyte, and the residence time of bacteria within the choanocyte. Preliminary analysis of the pumping rates of the three sponges studied here and the data of Kilian (1952) on spongillids indicates a water turnover rate of 6–20 ml/ml fresh sponge/min. Choanocyte densities of 6×10^8/ml of tissue for *Microciona prolifera* (Reiswig, unpublished) indicate a filtration rate of approximately $1.4–4.8 \times 10^{-5}$ ml/choanocyte/day. At bacterial concentrations found in Jamaica (5×10^4 cells/ml), bacterial availability is only 0.7–2.4 cells/choanocyte/day. Since residence time for particles within choanocytes is very short, probably much less than 3 hours (van Weel, 1949), accumulations of extranuclear DNA above normal background level are not expected to be detectable under natural conditions.

Efficient particle filtration in the bacterial size range, 1 μ, has been demonstrated in several other metazoan phyla (Jørgensen, 1949, 1966). The extensive studies on particle filtration in oysters, summarized in the study of particle retention in *Crassostrea virginica* by Haven and Morales-Alamo (1970), indicate that a single particle capture system is operative in these organisms. At particle sizes below 3–4 μ, retention efficiency falls rapidly in *C. virginica*, as found here in the primary amoebocyte system of sponges. In the bacterial size ranges encountered in this study 0.3–0.6 μ, retention efficiencies in *C. virginica* are less than 20%, far below the 96% level found in these sponges. The general lack of bivalves and ascidians in the outer reef habitat of Jamaica may be attributable to several factors. Species with high pumping efficiency (see Jørgensen, 1966, for tabulized summary) employ ciliary filtration (*e.g., Crassostrea*), do not utilize particles in the

bacterial size range, and are probably thus unable to retain the URPOC material available in Jamaican waters. Species demonstrating high retention in the bacterial size range (*e.g., Mytilus*) employ mucous sheet filtration, and exhibit lower pumping efficiencies due to the higher "cost" of propelling water through mucous sheets. These species, although probably able to utilize significant portions of the URPOC, would be unable to meet the higher cost of lower pumping efficiency (tabulated by Jørgensen, 1966) in the relatively nutrient-poor waters of the outer coral reefs. The Porifera, strikingly specialized for efficient retention of small particles including the URPOC fraction, and exhibiting high pumping efficiencies, are apparently able to maintain their role as dominant filter feeders in coral reef situations, free of significant competition from other filter feeding taxa.

It has been shown that the choanocyte capture system supplies approximately 81.4% (URPOC + bacteria) of the total POC diet of these relatively highly organized demosponges. In less complex and presumably primitive Porifera (Calcarea, Hexactinellida, and some homosclerophorid Demospongiae) choanocytes often comprise a greater proportion of the biomass of the sponge than all other cell types. If choanocyte function is uniform, and it appears so in fresh-water and marine demosponges, the less complex sponges may be essentially restricted to the 0.1–1 μ particle size fractions. It is hypothesized that in these simple sponges little or no transfer of particles from choanocytes to amoebocytes occurs, but instead assimilation presumably takes place within individual choanocytes. Bacteria and URPOC material may thus have been the original food source of Porifera, while evolution of amoebocyte cycles later allowed the phylum to utilize larger planktonic fractions, invade temperate coastal habitats, and develop more complex, denser and larger body masses. The evidence for asconoid morphology of early Cambrian hexactinellid and heteractinid sponges (Finks, 1970) is consistent with this hypothesis. The full development of amoebocyte cycles and thick body walls has taken place almost exclusively in the Demospongiae and allowed radiation of the class throughout the continental shelves and shallow seas of the world. The generally thin-walled Hexactinellida and Calcarea, hypothesized to be almost exclusively limited to the primitive choanocyte feeding system, have presumably been unable to utilize the larger sized plankton fractions, and are thus restricted in numbers of species, form-diversity and habitat.

I wish to express my deep appreciation for the aid and continued encouragement provided by Dr. W. D. Hartman and the late Prof. T. F. Goreau throughout this study. I also thank Prof. G. E. Hutchinson, Prof. T. H. Waterman, Dr. D. C. Rhoads and Dr. K. S. Thomson for discussion of the material and suggestions in preparation of the manuscript; and the students and staff at the Discovery Bay Marine Laboratory for their invaluable assistance in carrying out both field work and laboratory analyses.

Summary

1. Microscopic and chemical analysis of ambient and exhalant water samples collected *in situ* indicates that the net POC diet of three tropical Demospongiae, *Mycale* sp., *Verongia gigantea* and *Tethya crypta*, consists primarily (80.5%) of

particulate (filterable) organic matter which is unresolvable by direct microscopy (URPOC). Microscopically resolvable particulate material (MPOC) accounts for only the remaining 19.5% of the POC diet of these sponges.

2. The three sponges retain available resolvable particulate material within the size range of 0.3–50 μ at high efficiencies—means are 79.0% by calculated carbon content and 82.0% by particle volume.

3. Major components of the MPOC diet and of the total POC diet of these sponges are respectively: unarmored cells 83%, 16.2%; armored cells 12.3%, 2.4%; and bacteria 4.6%, 0.9%.

4. Two functionally independent capture systems appear to be operative in all 3 species, accounting for a basic bimodal pattern of particle retention. A system involving particles between 5 and 50 μ is attributable to phagocytosis by cells lining the inhalant system. A second system involving particles of the bacterial size range (0.3–1 μ) involves capture at the choanocyte collar and ingestion by choanocytes.

5. The amoebocyte capture system is by necessity constantly functional and must accept all particles entering the ostia. A transport path by amoebocyte migration cycles is proposed to account for transport and release of intact armored plankton at significant rates.

6. Bacterial retention is high in all species, 94.8–96.9%, mean = 96.1% by cell number. This choanocyte capture system is fallible, but under normal environmental conditions retention rates are constant and independent of ambient concentrations.

7. Sponges with high tissue density (*Verongia*) show apparent size selection of armored cells which is attributed to slow amoebocyte cycling. Sponges with low tissue density (*Mycale*) retain armored fractions at lower efficiencies without apparent size selection. High retention of armored cells by *Tethya* may be attributed to behavioral adaptation.

8. All three species effect a net production of microscopically resolvable detrital organic matter.

9. The previously unrecognized unresolvable fraction of particulate organic matter (URPOC) represents an available carbon source 7 times that of all resolvable planktonic material in Jamaican waters. The ability of sponges to capture this material, probably via the primitive choanocyte system, is responsible for continuing dominance of Porifera as the filter feeders of coral reef habitats.

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